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The Surfactant Tween 80 Enhances Biodesulfurization▽†

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In biocatalytic conversions, substrates and products may display inhibitory or toxic effects on the biocatalyst. *Rhodococcus erythropolis* 1awq could further remove sulfur from hydrodesulfurized diesel oil, and the biodesulfurization was enhanced by the surfactant Tween 80. Tween 80 was shown to decrease the product concentration associated with the cells, reducing product inhibition.

Most fossil fuels contain organic sulfur compounds. When combusted, SO_2 is released, resulting in serious pollution, e.g., acid rain (11, 22). The Environmental Protection Agency of the United States has proposed the reduction of the accepted sulfur level of diesel oil from 500 ppm to 15 ppm by 2006 (1). Presently, biodesulfurization is being investigated intensively because of its low cost, mild reaction conditions, and low impact on the environment (5, 13, 15, 16, 21, 37). With dibenzothiophene (DBT) as the model compound (5, 13, 21–23), research has been focused on strains that can selectively remove sulfur by converting DBT to 2-hydroxybiphenyl (2-HBP), known as the "4S" pathway. This conversion has been observed in *Rhodococcus* sp. (3, 6, 8, 24, 35, 36), *Paenibacillus* sp. (13), *Pseudomonas* spp. (29, 31), *Corynebacterium* sp. (25), and *Mycobacterium* sp. (15, 16). The research on biodesulfurization over the past years has been reviewed previously (12, 34).

It is known that surfactants can promote the solubility of hydrophobic substances in water (4, 32). Among various biocatalytic conversion methods, biodesulfurization is a reaction in the two-phase (oil-water) system. Polyethylene glycol sorbitan monooleate (Tween 80) is a nonionic surfactant as well as an oil-in-water emulsifier. Although the effects of surfactants on the degradation of hydrophobic compounds have been studied, its role in biodesulfurization has not been investigated to the best of our knowledge. The present investigation was designed to demonstrate the role of Tween 80 in aqueous and biphasic biodesulfurization processes.

The effect of Tween 80 on the cell growth of a desulfurization strain was studied. The strain 1awq used in this study, previously suggested as a *Rhodococcus* species, has been shown to selectively degrade DBT via the "4S" pathway (17, 33). It was further identified as *R. erythropolis* based on the conventional markers and the chemotaxonomic results (see Table S1 in the supplemental material). As a control, strain 1awq was cultured in basal salts medium (BSM) (36, 37) without surfactant. Dibenzothiophene (0.5 mM) was the sulfur source for growth. Compared to the cell yield of the control (2.5 g dry cell weight liter^{-1}), the biomass after 42 h incubation showed a trend of increasing with Tween 80 (Fig. 1). Cells with a concentration of 3.5 g liter^{-1} were obtained when 0.4% Tween 80 was added. According to the results of surface tension measurement (32), there were no surfactant-like molecules produced in the culture of strain 1awq grown in BSM. As shown in Table 1, strain 1awq grew poorly with Tween 80 as the sole sulfur source, which indicated there were no available sulfur impurities in Tween 80. About 0.2 mM of 2-HBP was produced in the control experiment (Fig. 1). This was the level that could fully inhibit cell growth (33). Hence, there was no further increase in the production of 2-HBP. With the addition of Tween 80, the concentration of 2-HBP went up. The highest concentration of 2-HBP (0.4 mM) was detected at a Tween 80 concentration of 0.4%.

The role of Tween 80 in biodesulfurization was studied. The critical micelle concentration (CMC) is the concentration at which the surfactant molecules saturate the solution and form micelles upon further addition of surfactant (2, 18). The CMC of the surfactant Tween 80 was determined by measuring the change of surface tension. The CMC value of Tween 80 in 0.1 M phosphate buffer (pH 7.0) with 8 g liter^{-1} of strain 1awq cells was between 0.3% and 0.35% (Fig. 2). Biodesulfurization activity was estimated by measuring the concentration of 2-HBP produced from DBT by use of high-performance liquid chromatography (Agilent 1100 series; Hewlett-Packard). With Tween 80, the desulfurization activity was increased. When the concentration of Tween 80 was above the CMC, the highest activity was reached at about 1 μmol g^{-1} min^{-1}. This activity was 35% higher than that seen without Tween 80 (data not shown). Typically, *Rhodococcus* sp. has a high affinity for the oil-water interface due to the hydrophobicity of its cell wall (23, 30). In this study, 0.3 mM 2-HBP was added to 0.1 M phos-

TABLE 1. Effect of Tween 80 on cell growth

Culture	Biomass (g liter^{-1}) at:	
	0 h	42 h
BSM without sulfur source	0.034 ± 0.0003	0.333 ± 0.049
BSM with 0.3% Tween 80	0.035 ± 0.0003	0.313 ± 0.024
BSM with 0.7% Tween 80	0.036 ± 0.002	0.398 ± 0.083
BSM with 0.5 mM DBT	0.035 ± 0.006	2.508 ± 0.295

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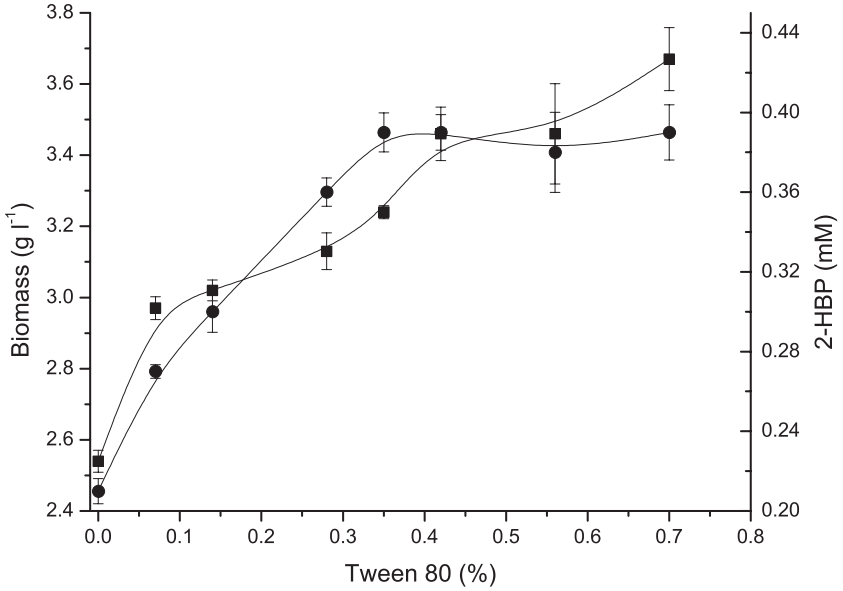

FIG. 1. Effects of different concentrations of Tween 80 on cell growth and 2-HBP production. Symbols: ■, biomass; ●, 2-HBP concentration. DBT was dissolved in *N*,*N*-dimethylformamide (DMF).

phate buffer (pH 7.0) with 8 g liter^{-1} of strain 1awq cells. After adequate mixing at 4°C, the cells absorbed 0.16 mM 2-HBP. With the addition of Tween 80, the concentration of 2-HBP in the supernatant was increased (Fig. 2). Therefore, we concluded the micellar solution of Tween 80 and oil-soluble compounds reduced the concentration of 2-HBP around the cells, which accounts for the increase in the desulfurization activity as well as in the production of biomass and 2-HBP (see Fig. S1 in the supplemental material). Considering the hydrophobic nature of *Rhodococcus* sp. (23, 30), the role of Tween 80 in the biphasic reaction was, for the first time, identified as the formation of a micellar solution with hydrocarbon to which the microorganisms did not have direct access.

Further investigation was carried out to demonstrate that Tween 80 could enhance diesel oil desulfurization. Cells with high biodesulfurization activity were harvested and tested in an FHD200 diesel oil system. FHD200, a hydrodesulfurized diesel oil, was provided by the Fushun Research Institute of the Sino Petroleum & Chemical Corporation. The sulfur content of the diesel oil was 200 ppm. The biocatalyst was suspended in 0.1 M

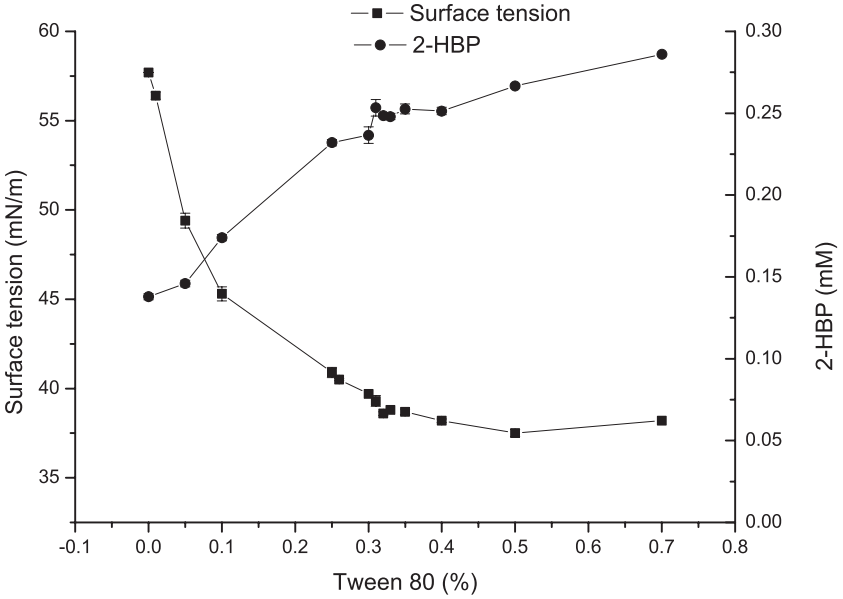

FIG. 2. The role of Tween 80 in biodesulfurization. Values represent surface tension determinations for biocatalysts and 2-HBP concentrations in the supernatant with different concentrations of Tween 80. The CMC of the surfactant Tween 80 in 0.1 M phosphate buffer (pH 7.0) with 8 g liter^{-1} of strain 1awq cells was from 0.3% to 0.35%.

TABLE 2. Effect of Tween 80 on diesel oil desulfurization of FHD200

% Tween 80	Total sulfur after biodesulfurization (ppm)	% Effectiveness of diesel oil desulfurization
0	46.5	76.8
0.18	21.8	89.1
0.35	19.3	90.4
0.50	17.8	91.1
0.70	18.6	90.7
1.05	17.7	91.2

phosphate buffer (pH 7.0) to reach a concentration of 8 g (dry cell weight) liter^{-1} and supplemented with hydrodesulfurized diesel oils (oil-to-water ratio of 1:9) (17). The cultures were given 2% glucose as a carbon source and different concentrations of Tween 80, while a culture containing no Tween 80 served as the control. The effectiveness of diesel oil biodesulfurization was enhanced by the addition of Tween 80 (Table 2). This result was further corroborated with FHD406 and KHD168 diesel oil results (see Table S2 in the supplemental material), where the values of the effectiveness of diesel oil desulfurization when 0.5% Tween 80 was added were 78.1% and 65.0%, respectively, compared to 56.6% and 52.9% without Tween 80. The main sulfur-containing compounds in diesel oils FHD406 and KHD168 before and after treatment and with and without Tween 80 were determined by gas chromatography with an atomic emission detector (Agilent G2350A; Hewlett-Packard) fitted with a PONA column (50 m by 0.2 mm by 0.5 μm) (see Table S2 in the supplemental material). Figure S2 in the supplemental material shows chromatogram values obtained by gas chromatography with the atomic emission detector; many of the sulfur-containing compounds in diesel oil FHD406 were found (see Fig. S2a in the supplemental material). Some of the compounds could still be detected after biodesulfurization without Tween 80 (see Fig. S2b in the supplemental material), but almost none were detectable after treatment with the supplement of 0.5% Tween 80 (see Fig. S2c in the supplemental material). Kaufman et al. (9, 10) reported that in a biphasic system, the size of the emulsion in the emulsion phase contactor was different from that in the impeller-based reactor whereas the rates of DBT oxidation in both reactors were similar. Marcelis et al. (19) estimated that the mass transfer rate of DBT from within an oil droplet to the oil-water interface was higher by at least a factor of 10 and was up to 10^4 higher than experimentally determined specific DBT conversion rates. These observations imply that mass transfer is not a rate-limiting step for biodesulfurization. As for the biodegradation of diesel oil, many surfactants can enhance the degradation by increasing the hydrocarbon dissolution rate (7, 14, 20). Hence, it should be noted that the role of surfactants in biodesulfurization is different from that in the biodegradation of diesel oil.

Several interesting potential biocatalytic conversions involve hydrophobic substrates and products including heterocyclic compounds (28). In some cases, problems arise from substrates and products that are poorly soluble in water and/or display inhibitory or toxic effects on the biocatalyst (26, 27). The nonionic chemical surfactant Tween 80 can enhance the biodesulfurization activity in both aqueous and biphasic systems by reducing the concentrations of the products around the cells. Conversely, Tween 80 can also reduce the concentrations of hydrophobic substrates associated with the cells. As long as the concentrations support adequate reaction rates, this reduction will not limit the overall conversion. If a substrate is also inhibitory at high concentrations, the addition of Tween 80 is theoretically stimulatory. The phenomenon reported here is applicable to microorganisms with a relatively hydrophobic cell surface.

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